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# Promiscuous Affairs of PKB/AKT Isoforms in Metabolism

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## **Abstract**

The protein kinase B (PKB) family encompasses three isoforms; PKB $\alpha$  (AKT1), PKB $\beta$  (AKT2) and PKB $\gamma$  (AKT3). PKB $\alpha$  and PKB $\beta$ , but not PKB $\gamma$ , are prominently expressed in classical insulin-sensitive tissues like liver, muscle and fat. Transgenic mice deficient for PKB $\alpha$ , PKB $\beta$  or PKB $\gamma$  have been analysed to study the roles of PKB isoforms in metabolic regulation. Until recently, only loss of PKB $\beta$  was reported to result in metabolic disorders, especially insulin resistance, in humans and mice. However, a new study has shown that PKB $\alpha$ -deficient mice show enhanced glucose tolerance accompanied by improved  $\beta$ -cell function and higher insulin sensitivity in adipocytes. These findings prompted us to review the relevant literature on the regulation of glucose metabolism by PKB isoforms in liver, skeletal muscle, adipocytes and pancreas.

**Keywords:** PKB, AKT, redundancy, metabolism, glucose, liver, muscle, fat, pancreas

## Introduction

The level of circulating glucose has to be adjusted to variations in food intake and energy demands, which is primarily regulated by the insulin/glucagon system in mammals. Insulin lowers blood glucose by increasing uptake and deposition into muscle and adipose tissue as well as by decreasing its release from the liver. Glucagon mainly acts on hepatocytes where it opposes the action of insulin and stimulates release of glucose into circulation. Insulin signalling targets various cell types in the whole organism and, in addition to its role in metabolism, affects cellular processes such as protein synthesis, proliferation and survival. Therefore a complex network of molecular pathways is required to transduce insulin signalling into cell type-specific and context-dependent responses. Protein kinase B (PKB) has been shown to be a key element in the insulin signal transduction network.

The physiological and tissue-specific effects of the three PKB isoforms have been extensively studied *in vitro* but most comprehensively in transgenic mice. While *Pkbα*<sup>-/-</sup> and *Pkbγ*<sup>-/-</sup> mice show impaired foetal growth and brain development, respectively, glucose homeostasis was found unaffected in both models (Chen et al., 2001, Cho et al., 2001b, Easton et al., 2005, Tschopp et al., 2005, Yang et al., 2003). In contrast, *Pkbβ*<sup>-/-</sup> mice are insulin resistant, mildly glucose-intolerant and have less adipose tissue. Depending on strain and gender, these mice show either late loss of β-cells followed by development of diabetes and mild growth deficiency, or compensatory increase of β-cell mass without age-dependent progression into overt hyperglycaemia (Cho et al., 2001a, Garofalo et al., 2003). These studies suggested that only PKBβ plays a role in regulation of energy homeostasis. This view was recently challenged by a new study (Buzzi et al., 2010) that re-examined in parallel the metabolic phenotype of three mouse strains deficient for *Pkbα*, *β* or *γ*, respectively. Here they confirmed that *Pkbβ*<sup>-/-</sup> mice are insulin resistant with compensatory increase of islet mass and that *Pkbγ*<sup>-/-</sup> mice show no metabolic abnormalities. However, *Pkbα*<sup>-/-</sup> mice displayed improved insulin sensitivity, lower blood glucose and higher serum glucagon concentrations. These new findings prompted us to critically review the relevant literature on the metabolic role of PKB isoforms.

## Protein Kinase B

The PKB serine/threonine protein kinase family consists of three evolutionary conserved isoforms: PKB $\alpha$  (AKT1), PKB $\beta$  (AKT2) and PKB $\gamma$  (AKT3). PKB was first isolated from transforming murine leukemia virus AKT-8 by Staal et al in 1977, characterized as an oncogene and named *akt* (Staal et al., 1977). Two human homologues of the viral *akt* gene were identified later on and termed *akt1* and *akt2*. In 1991, a human serine/threonine kinase was cloned, which was termed *related to the A and C kinases* (RAC) and subsequently renamed to PKB $\alpha$ /Akt1 ((Jones et al., 1991), reviewed in (Brazil and Hemmings, 2001)). Since the identification of PKB $\alpha$  as a serine/threonine kinase, almost 20 years ago, PKB isoforms were studied intensively and are now considered as major regulators of elementary cellular process, such as proliferation, survival, cell growth and energy metabolism (Bozulic et al., 2008, Contreras-Ferrat et al., 2010, Haga et al., 2005, Heron-Milhavet et al., 2006). Consequently, PKB isoforms play pivotal roles in physiology and their deregulation engenders diseases, such as cancer, neurodegeneration and metabolic disorders (Altomare and Testa, 2005, Zhao and Townsend, 2009). Remarkably, activation of PKB isoforms by gene amplification or mutations in upstream regulators frequently occurs in human cancers (Carpten et al., 2007). The molecular and cellular biology of PKB isoforms has been comprehensively reviewed (Brazil et al., 2002, Hanada et al., 2004, Manning and Cantley, 2007) and is therefore discussed only briefly in this review.

PKB $\alpha$ , PKB $\beta$  and PKB $\gamma$  are encoded by three distinct genes, which are located on different chromosomes. In contrast to many members of other kinase families, PKB isoforms share the same protein structure and are approximately 80% identical at the amino acid level. They carry a N-terminal pleckstrin homology (PH) domain, a catalytic domain and a C-terminal regulatory domain (Hanada et al., 2004). PKB $\alpha$  and PKB $\beta$  are ubiquitously expressed, whereas PKB $\gamma$  expression is restricted to brain, testis, lung, fat, mammary glands and pancreatic islets (Buzzi et al., 2010, Yang et al., 2003). Low expression levels were also observed in skeletal muscle (Brozinick et al., 2003). Notably, expression of PKB $\alpha$  and PKB $\beta$  is prominent in

classical insulin target tissues involved in the regulation of systemic energy homeostasis, such as liver, skeletal muscle and fat (Yang et al., 2003).

PKB isoforms are activated by growth factors and cytokines, including PDGF, VEGF, HGF, IGF-1, insulin,  $\text{TNF}\alpha$  and IL-2 but also by environmental stresses, such as heat shock, hypoxia and oxidative stress (Zhuravleva et al., 2010). Regulation of PKB downstream of these stimuli generally depends on activation of phosphatidylinositol 3-kinase (PI3K) family members, which convert phosphatidylinositol di-phosphate (PIP<sub>2</sub>) to PIP<sub>3</sub> at the plasma membrane. PKB isoforms bind to PIP<sub>3</sub> via their PH domain, which facilitates their activation. PKB isoforms are phosphorylated and thereby activated by upstream kinases at two distinct phosphorylation-sites. First, PDK1 phosphorylates PKB isoforms at the catalytic domain (Thr308 in PKB $\alpha$ , Thr309 in PKB $\beta$  and Thr305 in PKB $\gamma$ ) which results in basal kinase activity of approximately 10%. In a second step, PKB isoforms are phosphorylated by mTORC2, DNA-PK or ATM at the C-terminal regulatory domain (Ser473 in PKB $\alpha$ , Ser474 in PKB $\beta$  and Ser472 in PKB $\gamma$ ), which is essential for full kinase activity. Activation of PKB isoforms is tightly counter-regulated by phosphatases, such as PTEN and SHIP2. These phosphatases inactivate PIP<sub>3</sub> by dephosphorylation and thereby prevent plasma membrane translocation and activation of PKB isoforms (Bhaskar and Hay, 2007, Brazil and Hemmings, 2001, Hanada et al., 2004, Zhuravleva et al., 2010).

Activity of PKB isoforms is modulated by different binding partners, such as TRAF6, HSP90, CTMP and TCL1 which affect protein stability, dynamics and duration of activation and rate of kinase activity, respectively (Brazil et al., 2002). Furthermore, recent studies show that activity of PKB isoforms is indirectly modulated by several microRNAs. For instance, miR320 was shown to down-regulate PI3K in adipocytes resulting in inhibition of PKB activation and insulin resistance (Ling et al., 2009). However, it remains to be determined if modulation of PKB activity by binding partners and microRNAs plays a role in insulin signalling and regulation of glucose homeostasis.

Upon activation, PKB isoforms are released from the plasma membrane and phosphorylate various substrates throughout the cell. PKB substrates can specifically regulate a single respective cellular process (e.g. cell survival, Bad or caspase 9), or pleiotropically affect several cellular functions simultaneously, such as Gsk3 $\beta$  and

FoxO transcription factors which can control cell survival, proliferation but also energy metabolism (Manning and Cantley, 2007)

The different phenotypes of *Pkbα*<sup>-/-</sup>, *Pkbβ*<sup>-/-</sup> and *Pkbγ*<sup>-/-</sup> mice undoubtedly point to isoform-specific functions. As the expression of PKB isoforms overlaps in many organs, the different phenotypes can not solely be explained by divergent gene expression. There is an emerging number of studies describing isoform-specific functions in cellular processes, such as PKBα in cancer cell migration (Chin and Toker, 2010), PKBα in β-cell proliferation and PKBβ in glucose uptake. Since PKB isoforms are structurally highly similar, including the kinase domain, it is unlikely that recognition of phosphorylation-motifs underlies substrate-specificity (Manning and Cantley, 2007). Therefore it is considered that substrate-specificity is controlled by cellular localization and specific binding partners. However, until today, functional differences of PKB isoforms at the cellular level are not yet fully characterized and the mechanisms determining substrate-specificity remain largely unknown.

## Liver

The liver functions as a critical regulator of glucose homeostasis and the PI3K/PKB pathway co-ordinates hepatic glucose metabolism with the systemic metabolic state. Out of the three PKB isoforms, only PKBα and PKBβ, but not PKBγ, are expressed in the liver. Studies using transgenic mice have shown, that PKBβ accounts for approximately 70% of total PKB protein in the liver and is therefore considered as the major isoform (Dummler et al., 2006). In hepatocytes, PKBα and PKBβ are both activated upon insulin stimulation in a PI3K-dependent manner (Taniguchi et al., 2006).

According to the current model, insulin suppresses hepatic glucose output in several ways, including inhibition of gluconeogenesis and stimulation of glycogen synthesis, which are both dependent on PKB activity (Newgard, 2003). Gluconeogenesis is suppressed after phosphorylation/inhibition of the transcription factor FoxO1, an inducer of gluconeogenic genes *pepck* and *g6pase* (Taniguchi et al., 2006). In addition, PKB also induces glycogen synthesis by phosphorylating and inhibiting GSK3β (Lawrence and Roach, 1997). Although it was shown that PKBβ (He et al.,

2010, Leavens et al., 2009) plays a critical role in these processes, the effects of endogenous PKB $\alpha$  remain unclear. Furthermore, it was proposed that hepatic *de novo* lipogenesis is mainly regulated by PKC $\lambda/\zeta$ , as expression of lipogenic genes, such as *srebp-1c*, is depended on PKC $\lambda/\zeta$  activity (Taniguchi et al., 2006). Even so, several studies show that PKB $\alpha$  and PKB $\beta$  promote hepatic *de novo* lipogenesis as well (He et al., 2010, Leavens et al., 2009, Ono et al., 2003).

The role of PKB activity in hepatic metabolism and its effects on systemic energy homeostasis was studied in mice with liver-specific deletions of major regulatory subunits of PI3K (*pik3r1<sup>Δli</sup>/pik3r2<sup>Δli</sup>*) and PTEN (*pten<sup>Δli</sup>*) (Horie et al., 2004, Stiles et al., 2004, Taniguchi et al., 2006). Insulin-stimulated activation of PKB $\alpha$ , PKB $\beta$  and PKC $\lambda/\zeta$  was almost completely abrogated in *pik3r1<sup>Δli</sup>/pik3r2<sup>Δli</sup>* mice. Concomitantly, insulin also failed to downregulate hepatic gluconeogenesis and could no longer inhibit GSK3 $\beta$  and induce expression of lipogenic genes. As a consequence, *pik3r1<sup>Δli</sup>/pik3r2<sup>Δli</sup>* mice exhibited insulin resistance, hyperglycemia, hyperinsulinaemia and were glucose intolerant (Taniguchi et al., 2006). Remarkably, expression of gluconeogenic genes, *pepck* and *g6pase* was efficiently blocked after overexpression of constitutive active PKB $\alpha$  (myr-PKB $\alpha$ ) whereas expression of lipogenic genes, *srebp-1c*, could only be restored by overexpression of constitutive active PKC $\lambda/\zeta$  (Taniguchi et al., 2006). On the other hand, gluconeogenic genes were down regulated and phosphorylation of GSK3 $\beta$  and lipogenesis were enhanced in liver of *pten<sup>Δli</sup>* mice, most likely due to hyper-activated PKB $\alpha$ , PKB $\beta$  and PKC $\lambda/\zeta$ . As a result, *pten<sup>Δli</sup>* mice were found to be hypoglycaemic, hypoinsulinaemic, showed increased glucose tolerance and, most strikingly, developed hepatic steatosis with all characteristics of human non-alcoholic fatty liver disease (Horie et al., 2004, Stiles et al., 2004).

In recent studies the role PKB $\beta$  in hepatic lipid accumulation was examined using different mouse models of hepatic steatosis (He et al., 2010, Leavens et al., 2009). Remarkably, whole-body deletion of *pkb $\beta$*  in *pten<sup>Δli</sup>*, leptin-deficient (*lep<sup>ob/ob</sup>*) and mice on high-fat diet (HFD) as well as liver-specific deletion of *pkb $\beta$*  in *lep<sup>ob/ob</sup>* and mice on HFD significantly reduced lipid accumulation in hepatocytes. PKB $\beta$ -deficiency reduced expression of lipogenic genes and *de novo* lipogenesis, indicating that PKB $\beta$  is required for lipid accumulation in hepatocytes.



Interestingly, *pkbβ*-deficiency had more pronounced effects in wildtype controls compared to *pten<sup>Δli</sup>* mice. Therefore, increased activation of PKB $\alpha$ , and possibly also of PKC $\lambda/\zeta$ , might compensate for loss of PKB $\beta$  in hepatocytes. Indeed ectopic expression of constitutively active PKB $\alpha$  (myr-PKB $\alpha$ ) in the liver induced hypoglycemia and hepatic steatosis, further supporting the notion for functional overlap between PKB $\alpha$  and PKB $\beta$  in hepatocytes (Ono et al., 2003). However, activation of PKB isoforms by myristilation is rather artificial, and might induce non-physiological functions.

Notably, hepatic lipid content, but not de novo lipogenesis or expression of lipogenic genes, were reduced in PKB $\beta$ -deficient mice fed a specific HFD (*Surwit diet*, (Leavens et al., 2009)). Moreover, expression of myr-PKB $\alpha$  upregulated *srebp-1c* in wildtype but not *pik3r1<sup>Δli</sup>/pik3r2<sup>Δli</sup>* mice and additionally promoted accumulation of hepatic lipids independent of *srebp-1c* (Ono et al., 2003, Taniguchi et al., 2006). These observations suggest that PKB $\alpha$  and PKB $\beta$  regulate other processes in addition and that reduced lipogenesis could be a secondary effect dependent on PI3K/PKC.

### **Skeletal muscle.**

Skeletal muscle is a specialised tissue that makes movement possible by transforming chemical energy into mechanical force. In addition, skeletal muscle is also central to metabolic regulation and 70-90% of glucose disposal during a hyperinsulinemic euglycaemic clamp occurs in this tissue (DeFronzo et al., 1981). Glucose taken up is mainly incorporated into glycogen (Shulman et al., 1990) and the majority ( $\approx 80\%$ ) of carbohydrates stored in humans are found in skeletal muscle (Jensen and Lai, 2009).

It is the generally accepted view that insulin activates PKB via class 1A PI3K (Shepherd, 2005). Although this has not been conclusively shown in fully differentiated skeletal muscle, all available data support the view that PKB promotes insulin-stimulated glucose uptake and glycogen synthase activation (Cleasby et al., 2007). Like in adipose tissue, glucose uptake is increased in skeletal muscle by triggering translocation of GLUT4 from intracellular vesicles to the plasma

membrane, which depends on well studied signalling events downstream of the insulin receptor involving IRS, PKB and AS160. Skeletal muscle expresses all three PKB isoforms (Brozinick et al., 2003, Turinsky and Damrau-Abney, 1999) but only deletion of PKB $\beta$  causes insulin resistance and reduces insulin-stimulated glucose uptake (Cho et al., 2001a, Garofalo et al., 2003), indicating that PKB $\beta$  is required for this process. However, since high concentrations of insulin could still increase glucose disposal into muscle lacking PKB $\beta$  other signalling components might also be able to regulate GLUT4 translocation downstream of insulin. Indeed, overexpression of constitutively active PKB $\alpha$  increases glucose uptake in L6 muscle cells (Hajdich et al., 1998) suggesting that PKB $\alpha$  and PKB $\beta$  can both regulate translocation of GLUT4 to the plasma membrane. This finding is in line with the observation that insulin can activate all three isoforms of PKB in skeletal muscle (Brennesvik et al., 2005, Brozinick et al., 2003). Isoform-specific function has been investigated in several studies. Ectopic expression of constitutively active PKB $\alpha$  or PKB $\beta$  *in vivo* in rat muscle fibres increased glycogen accumulation, but only expression of PKB $\beta$  increased basal glucose uptake (Cleasby et al., 2007). However, only PKB $\alpha$  increased glycogen synthase kinase-3 $\beta$  (GSK3 $\beta$ ) phosphorylation. Knockdown of PKB $\beta$  in fully differentiated muscle fibres by electrotransfer of short hairpin (sh)- RNAs decreased insulin-stimulated glucose uptake suggesting isoform-specificity of PKB in regulation of glucose metabolism (Cleasby et al., 2007). Unfortunately, the role PKB $\alpha$  was not addressed in this study. Interestingly, Brozinick et al. (Brozinick et al., 2003) found that insulin-stimulated PKB $\beta$  activation was reduced in insulin resistant muscles whereas insulin-stimulated activation of PKB $\alpha$  and PKB $\gamma$  occurred normally. Evidence for isoform-specific signalling was provided by Bouzakri et al (Bouzakri et al., 2006). These authors could show that activation of PKB $\beta$  via IRS1 stimulates glucose uptake whereas IRS2-mediated activation of PKB $\alpha$  increases lipid synthesis.

Besides insulin, muscle contraction can also induce glucose uptake. Contraction-induced glucose transport depends on increased translocation of GLUT4 but the underlying mechanism depends on AMPK and not on PI3K. However, contraction can increase PKB phosphorylation and activity (Sakamoto et al., 2003, Whitehead et al., 2000), but contraction-mediated PKB phosphorylation remains below 10% of insulin-stimulated PKB phosphorylation (Whitehead et al., 2000). Contraction

increases activity of all isoforms, but PKB $\alpha$  activation is most pronounced (Sakamoto et al., 2002). The notion that PKB does not mediate contraction-stimulated glucose uptake is in line with the fact that the PI3K inhibitor wortmannin does not inhibit contraction-stimulated glucose uptake (Whitehead et al., 2000). Accordingly, contraction-stimulated glucose transport is normal in skeletal muscle of PKB $\beta$ -deficient mice (Sakamoto et al., 2006). Interestingly, contraction can block insulin-stimulated class 1A PI3K activity without reducing insulin-stimulated PKB activation (Whitehead et al., 2000) indicating, that PKB phosphorylation might occur without increase in class 1A PI3K activity. This observation highlights the need to clarify which isoform(s) of PI3K mediate insulin-stimulated glucose uptake in skeletal muscle.

## **Adipose tissue**

Insulin induces phosphorylation of PKB in adipocytes. Like in muscle and liver, the level of phosphorylation/activation of PKB after insulin stimulation is often regarded as benchmark for insulin sensitivity. This consensus is based on observations, that insulin resistance in adipose tissue is in many cases associated with less insulin-induced phosphorylation of PKB and that increased or constitutive activation of PKB can increase or mimic insulin action, respectively. Many lines of evidence suggest, that mainly PKB $\beta$  is required downstream of insulin in adipocytes. For example, transient downregulation of PKB $\beta$  using siRNAs inhibits insulin-induced GLUT4 translocation to the plasma membrane of 3T3-L1 adipocytes whereas knockdown of PKB $\alpha$  did not result in any differences (Jiang et al., 2003, Katome et al., 2003). These findings are in line with the observation that *Pkb $\beta$* <sup>-/-</sup> mice are insulin resistant whereas *Pkb $\alpha$* <sup>-/-</sup> mice are normal or even more insulin sensitive (Buzzi et al., 2010, Chen et al., 2001, Cho et al., 2001a, Cho et al., 2001b, Garofalo et al., 2003, Yang et al., 2003). However, not all metabolic functions regulated by insulin in adipocytes might be dependent on PKB $\beta$  alone, as described by Katome et al. (Katome et al., 2003). These authors analysed 2-DG uptake and glycogen synthesis in 3T3-L1 adipocytes after downregulation of PKB $\alpha$ ,  $\beta$  or  $\gamma$  and found that PKB $\alpha$  and PKB $\beta$  contributed to insulin-stimulated glycogen synthesis to about the same extent while 2-DG uptake only depended on PKB $\beta$ . Accordingly, insulin appears to stimulate the

association of PKB $\beta$  with GLUT4-containing vesicles in rat adipocytes (Calera et al., 1998) and overexpression of PKB $\beta$ , but not PKB $\alpha$ , rescues impaired glucose transport in PKB $\beta$ -deficient adipocytes (Bae et al., 2003). How isoform-specificity might be achieved in adipocytes was studied by Gonzales and McGraw (Gonzalez and McGraw, 2009). These authors found that insulin activates both PKB $\alpha$  and PKB $\beta$  in adipocytes, but describe differential subcellular distribution of these two isoforms upon stimulation. While basally adipocytes had similar levels of PKB $\alpha$  and PKB $\beta$  at the plasma membrane, a significantly greater fraction of PKB $\beta$  accumulated at the plasma membrane after stimulation with insulin. The question if PKB plays a role in lipogenesis has received surprisingly little attention. However, Berggreen and colleagues (Berggreen et al., 2009) recently described that inhibition of PKB in 3T3-L1 adipocytes with an inhibitor called Akti reduced de novo and insulin-dependent lipid synthesis and that insulin failed to regulate the rate-limiting lipogenic enzyme acetyl-CoA carboxylase (ACC) when PKB was inhibited. Specific roles for the different isoforms of PKB were not described in this study.

Interestingly, there is a small but noteworthy number of studies with conflicting results. For example, Kitamura et al. (Kitamura et al., 1998) expressed a dominant-negative PKB isoform (Akt-AA) in 3T3-L1 adipocytes. This isoform contains two alanines instead of the two regulated phosphorylation sites (Thr308 and Ser473) and its expression reduced activation of PKB by about 80-95%. Expression of Akt-AA inhibited insulin-dependent protein synthesis without affecting glucose transport indicating, that PKB might only be required for some but not all effects of insulin in adipocytes. Similarly, Guilherme and Czech (Guilherme and Czech, 1998) presented evidence that the formation of IRS1/PI3K complexes and Akt/PKB activation are insufficient to stimulate glucose transport in rat adipocytes. At least two more recent studies also describe that insulin-dependent activation of PKB does not necessarily correlate with insulin-induced 2-DG transport (Hoehn et al., 2008, Xu et al., 2010). Finally, Buzzi et al. (Buzzi et al., 2010) found that primary adipocytes isolated from *Pkb $\alpha$* -deficient mice show higher insulin-induced glucose incorporation than adipocytes from wild type littermates.

## **Pancreatic islets**

To properly regulate blood glucose homeostasis islet mass and function has to be coordinated with metabolic demand. Plasticity of islet mass is achieved by integration of a complex signal environment comprised of nutrients, hormones and cytokines that controls the balance between apoptosis and cell growth/proliferation (Maedler, 2008, Niessen, 2006). Because PKB is a global regulator of growth, proliferation and apoptosis, it has been implicated to play a major role in modulating plasticity of islet mass downstream of insulin receptor substrate 2 (IRS2) (Elghazi et al., 2007, Hennige et al., 2003, Kubota et al., 2000, Lingohr et al., 2003, Mohanty et al., 2005, Park et al., 2006, Takamoto et al., 2008, Withers et al., 1998, Wrede et al., 2002). Yet, a number of studies addressing this issue yielded somewhat unexpected results. *Pkb $\alpha$* -deficient mice show impaired placental development and foetal growth (Buzzi et al., 2010, Chen et al., 2001, Tuttle et al., 2001, Yang et al., 2003) but normal islet growth and function. *pkb $\beta$* -deficient mice show impaired overall growth but display, dependent on strain and sex, even compensatory increase in  $\beta$ -cell mass (Buzzi et al., 2010, Cho et al., 2001a, Garofalo et al., 2003). Finally, *pkb $\gamma$* -deficient mice display reduction in brain size without any distortions of islet function or mass (Buzzi et al., 2010, Easton et al., 2005, Tschopp et al., 2005). In another study  $\beta$ -cell-specific loss of function for PKB was induced by expression of a kinase-dead dominant-negative form of PKB $\alpha$  (*rip-kdpkb*), however, only defective insulin secretion but no reduction in islet size was observed (Bernal-Mizrachi et al., 2004). Since the dominant-negative form antagonizes all three isoforms this latter finding makes compensation between isoforms an unlikely explanation for the normal islet phenotypes of PKB-deficient mice. In contrast to the loss of function phenotype, ectopic expression of constitutively active PKB $\alpha$  under the control of the rat insulin promoter (*rip*) (Bernal-Mizrachi et al., 2001, Tuttle et al., 2001) resulted in hypertrophy and hyperplasia of islets. Such mice were hyperinsulinaemic and resistant to streptozotocin-induced diabetes. Taken together the results from these mouse models suggest that although none of the PKB isoforms is required for maintenance of islet mass, constitutive activation of at least PKB $\alpha$  is sufficient to increase islet size. In order to reconcile these observations it was proposed (Niessen, 2006) that maintenance and compensatory expansion of islet mass (as observed in insulin resistance) do not depend on the same signal transduction pathways downstream of IRS2. This model predicts that PKB is only required for expansion but not for maintenance of islet mass. Which of the three PKB isoforms is/are required to

regulate islets mass was studied recently by Buzzi et al. (Buzzi et al., 2010). This study shows that only PKB $\alpha$ , but not PKB $\beta$  or  $\gamma$ , is specifically activated downstream of IRS2 in  $\beta$ -cells. Furthermore, adenoviral overexpression of PKB $\alpha$  increased proliferation of  $\beta$ -cells while overexpression of the remaining two isoforms was ineffective, indicating that PKB $\alpha$  is in control of the regulation of  $\beta$ -cell mass.

## **Perspectives and conclusions**

Insulin sensitivity manifests at the signalling level and at the level of cellular function. Since the insulin receptor is present on many, if not all, mammalian cells the biological function of insulin is cell type-specific, and, within a given cell type, insulin often controls more than one cellular process. For example, insulin induces GLUT4-dependent transport and deposition of glucose into fat. It also inhibits lipolysis. The analysis of any of these endpoints after stimulation with insulin allows unambiguous determination of how insulin sensitive the target cell is for the respective function. It has become common practice in the field to correlate insulin-induced cellular effects with intra-cellular insulin signal transduction, however, insulin sensitivity at the signalling level is not easy to measure because the insulin receptor connects to an intricate and highly context-specific intra-cellular network of signalling molecules. In practice insulin-dependent activation of few protein kinases within this network is usually correlated with specific insulin-induced cellular responses. PKB is regarded as most important mediator of metabolic insulin action and its activation is often monitored by using phospho-specific antibodies in combination with Western blotting. However, as described in this review, insulin signalling might branch at the level of PKB isoforms to control different aspects of metabolic regulation but in most of the cases, the possibility for non-redundant roles of PKB isoforms is not taken into consideration. Technically, due to high conservation of the aminoacid sequence surrounding the phosphorylated Ser and Thr residues in the three isoforms, none of the available phospho-specific antibodies can be used to determine which isoform(s) is(are) activated without prior isoform-specific immunoprecipitation. As more and more evidence for specific and possibly even opposing roles of PKB isoforms accumulates it appears justified to reconsider the appropriateness of detecting PKB phosphorylation to assess overall insulin sensitivity without consideration of the specific isoform.



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## **Declaration of interest**

The authors report no declarations of interest.

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